IMMUNOCYTOCHEMICAL STUDY OF CB1 RECEPTORS IN RAT'S PERIAQUEDUCTAL GRAY AFTER COLD STRESS AND EFFECTS OF PEPTIDES TYR-W-MIF-1 AND TYR-K-MIF-1

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ABSTRACT

The immunohistochemical localization of cannabinoid type 1 (CB1) receptors in periaqueductal gray (PAG) of male rats after acute cold stress and effects of endogenous antiopiate peptides Tyr-W-MIF-1 and Tyr-K-MIF-1 on nociception was studied. The nociception was measured by the paw pressure test. As control were used intact rats. Stress activates PAG as an important component of the descending inhibitory pain pathway and stress-induced analgesia. CB1 immunoreactivity appeared as puncta and was found in cell bodies, axons and dendrites. The morphometric analysis revealed that acute cold stress increases the density of CB1-immunoreactive neurons in PAG compared with expression in intact animals. Secund, the results showed that Tyr-K-MIF-1 and Tyr-W-MIF-1 decreased the density of CB1-immunoreactive neurons in PAG of control rats, acute cold stress and after acute cold stress and effects of endogenous antiopiate peptides Tyr-W-MIF-1 and Tyr-K-MIF-on nociception.

Key words: *CB1 receptors, PAG, cold stress, Tyr-K-MIF-1, Tyr-W-MIF*

INTRODUCTION

Stress is defined as a state of menaced homeostasis, evoking physiologically and behaviorally adaptive respons of the organism. The adaptive response reflects the activation of specific central circuits and is genetically and constitutionally programmed and permanently modulated by environmental factors. The adaptive responses in due to an acute stressor include the physiological and behavioral processes that are essential to reestablish homeostatic balance (1,2).

Cold stress provokes variable endocrine, physiological and behavioral responses by activating of several brain areas and pathways. The lateral subdivision of the medial preoptic nucleus and the median preoptic nucleus are accepted to be the center of of thermoregulation in the forebrain, but little is known about the descending loop of the cold evoked stress pathway (2).

The PAG is involved in the control of many aspects of stress and can influence the nociceptive sensory transmission and the integration of behavioral responses to stressful stimuli. Acute stress models have been reported to inhibit pain sensation by activating the brain pathways that project from the amygdala to the PAG, descend to the brainstem ventromedial medulla and dorsal horn of the spinal cord and engage opioid or non-opioid mechanisms.

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This adaptive response is referred to as stress-induced analgesia (SIA) (4). A number of findings indicate that PAG contributes to the enhanced antinociception produced by co-administration of opioids and cannabinoids (5).

One of the mechanisms known to play a part in the response of an organism to stress is activation of the endocannabinoid system in stress-responsive circuits (6,7). The endocannabinoid system is a signalling system, comprising of the endocannabinoids, which bind to a family of G-protein-coupled receptors, called CB1 and CB2 (8). CB1 receptors in stress-responsive neural circuits may play a critical role in regulating neuroendocrine and behavioral responses to stress (8).

On the other hand, Tyr-W-MIF-1 and Tyr-K-MIF-1 are members of Tyr-MIF-1 family neuropeptides, which are able to inhibit of some forms of SIA and are candidates for opiate modulators (10).

Futhermore, in the light of the above data, the purpose of the present study was to examine the effects of neuropeptides Tyr-W-MIF-1 and Tyr-K-MIF-1 on CB1 expression in PAG after cold stress in rats.

MATERIALS AND METHODS

Animals

The experiments were carried out on male (n = 6) Wistar rats (180-200 g) kept under normal conditions at ambient room temperature (22°C). Each group (control and experimental) included three rats.

Acute model of cold stress

The animals were placed in a refrigerating chamber at 4°C for 1 hour. The control group was not submitted to 1 hour stress procedure.

Drugs and treatment

Tyr-W-MIF-1 and Tyr-K-MIF-1 (both in dose 1 mg/kg) was obtained from Sigma. The neuropeptides were dissolved in sterile saline (0.9% NaCl) solution and were injected intraperitoneally (i.p).

The nociception was measured by means of the paw pressure test.

Immunocitochemistry

After the completion of the stress model, rats were anaesthetized with Thiopental (40 mg/kg, i.p.). Transcardial perfusion was done with 4% parafomaldehyde in 0.1 M phosphate buffer, pH 7.2. The brains were removed from skulls and postfixed for 1 hour in the same fixative at 4°C. Coronal sections were cut on a freezing microtom (Reichert-Jung) at 40 μm. Two series of sections taken at levels of bregma - 5.3 to - 8.3 mm from the bregma were selected (11). Free-floating sections were preincubated for 1 h in 5% normal goat serum in PBS. Afterwards, incubation of the sections was performed in a solution of the primary antibody for 48 h at room temperature. We used a polyclonal anti-CB1 antibody (the epitope, corresponding to amino acids mapping at the N-terminals of CB1, Santa Cruz, USA), in a dilution of 1:1000. Then sections were incubated with biotinylated rabbit polyclonal IgG (dilution, 1:500) for 2 h and in a solution of avidin-biotin-peroxidase complex (Vectastain Elite ABC reagent; Vector Labs., Burlingame CA, USA; dilution 1:250) for 1 h. This step was followed by washing in PBS and then in 0.05 M Tris-HCl buffer, pH 7.6, which preceded incubation of sections in a solution of 0.05% 3,3`-diaminobenzidine (DAB, Sigma) containing 0.01% H_2O_2 for 10 min at room temperature for the visualization.

Morphometric analysis was performed by capturing images of PAG through a 40 objective using a microanalysis system Nikon photomicroscope ECLIPSE 80i (digital camera DXM 1200C and the measured area of 0.360185 mm^2). Data the entire drawings were entered.

RESULTS AND DISCUSSION

The principal findings were as follows. First, the CS was found to modify the pain sensitivity and increased statistically significantly the pain threshold in animals compared to controls. The nociception was measured by means of the paw pressure test.

Second, immuno-staining patterns on coronal sections of PAG at levels of +5.3 to + 8.3 mm from bregma (11); were analyzed. It is clear from this study that CB1 receptors appears as punctata in axons, dendrites as well as around the perikarya in the all division of the PAG (Fig. 1). The immunoreactivity suggesting that the CB1 receptor is either primarily associated with discrete organelles in perikarya, or the label forms clusters on the cell membrane. The

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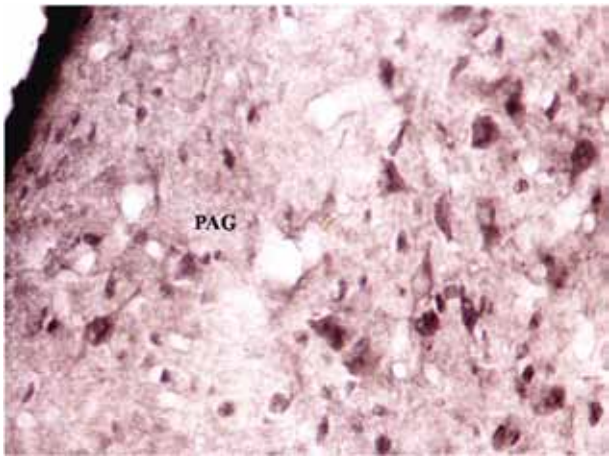

Fig. 1. CB1 – immunopositive neurons in the PAG of control animals. X 400

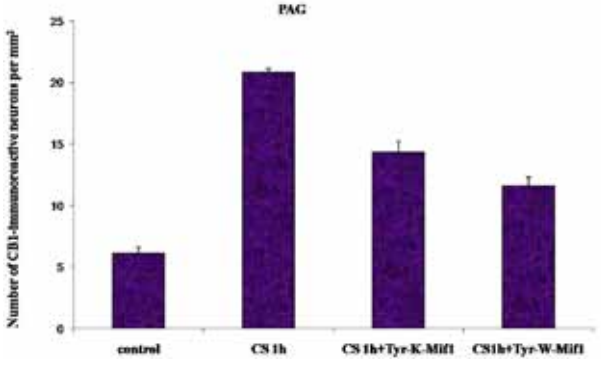

Fig. 2. The number of CB1- immunopositive neurons per μm2 of control, after cold stress 1h (CS 1h), in animals exposed to 1h CS and injected with Tyr-K-MIF-1 (CS 1h + Tyr-K-Mif1), and Tyr-W-MIF-1((CS 1h + Tyr-W-Mif1). Values are presented as means ± S.E.M

immunostaining of the CB1 immunoreactivity showed a striking specific pattern of neuronal profiles in PAG of control rats and after cold stress. The distribution of the CB1 immunoreactive neuronal elements generally coincided with that observed in previous studies that employed autoradiography, in situ hybridization and immunocytochemistry in CNS (12).

Third, morphometric analysis revealed that CS exposure increased the density of CB1 receptors in the PAG comparing with control rats. Introduced after 1h CS, both endogenous Endogenous opioid peptides CB1 - immunoreactive neurons in the PAG between control and acute colded rats (Fig. 2) and neuropeptides Tyr-K-MIF-1 and Tyr-W-MIF-1 decreased the expression of CB1-immunoreactive neurons in the PAG.

In conclution, our morphometric studies reveal differences in the density of CB1 – immuoreactive neurons in the rat PAG after acute CS 1h and treatment with endogenous opioid peptides -K-MIF-1 and Tyr-W-MIF-1. These new data may open the way to new studies in stress signalling in the PAG.

Financial disclosure

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